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Alterations in Gene Expression During Heat Shock of *Achlya ambisexualis*

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When exponentially growing cultures of *Achlya ambisexualis* strain E87 were raised from their normal growth temperature of 30°C to 35°C, the rates of synthesis of a small number of proteins were dramatically increased. The most predominant proteins synthesized in response to heat shock had molecular weights of 70,000 and 78,000, and their increased synthesis was detected as early as 10 min after the shift to 35°C. Changes in the populations of translatable messenger RNAs during heat shock showed that the levels of the mRNA's for all the major induced proteins correlated very closely with the alterations in the in vivo patterns, suggesting a transcriptional level of control of their synthesis. When after a period of heat shock (60 min) the cultures were shifted back to 30°C, recovery of the preshock patterns of protein synthesis was attained after several hours. Different proteins show temporally distinct patterns of recovery. During recovery the levels of translatable mRNA's for the induced proteins also correlated closely with the patterns of in vivo protein synthesis.

Heat shock responses have now been described in a number of organisms including *Drosophila* (1), several eucaryotic cell lines (11), cellular slime molds (4, 14), a protozoan (3), and some plants (2). For all these systems, a shift to elevated temperatures results in an increase in the rates of synthesis of a group of proteins. Some proteins, synthesized at a higher level before the heat shock, demonstrate a decreased rate of synthesis in all systems. Increased amounts of translatable RNAs coding for proteins induced by heat shock have been demonstrated in *Drosophila* (18) and *Saccharomyces* (16). In *Drosophila* this has been shown to be due to an increased rate of synthesis of heat shock-specific RNA (21) transcribed from puffs on polytene chromosomes induced by heat shock (17, 22).

Very little is known about the effect of heat shock on the filamentous fungi, a group of organisms which have been shown to have some interesting differences in their gene organization and expression when compared with higher eucaryotes (8–10, 13). *Achlya ambisexualis* (♂ strain E87) can be hormonally induced to undergo sexual differentiation with only subtle changes in gene expression (7, 21) and to undergo asexual reproduction with few detectable changes in gene expression (Gwynne and Brandhorst, submitted for publication). Thus, it was of interest to determine what changes in gene expression might be included in the physiological response to heat of this simple oomycete. We found that there were distinct, rapid changes in the synthesis of specific proteins and that these changes reflected corresponding changes in the levels of translatable mRNA's. Recovery from heat shock resulted in a return to the preshock patterns of synthesis after several hours.

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MATERIALS AND METHODS

Culture conditions. *A. ambisexualis* strain E87 (a gift from P. Horgen) was maintained in stock cultures on Emerson's YPSS agar (Difco Laboratories). Spores were produced by the method of Griffin and Breuker (5). PYG medium (100 ml; 0.125% Difco peptone, 0.125% Difco yeast extract, 0.3% dextrose) was inoculated with 2.5×10^5 spores, and the culture was shaken at 200 rpm for 24 h at 30°C (normal temperature). To induce heat shock cultures were shifted to 35°C. Recovery from heat shock involved a shift back to 30°C.

Labeling conditions. [^{35}S]methionine (20 μCi; 5 mCi/ml, 940 Ci/mmol; Amersham Corp.) was added to 1-ml samples withdrawn at specific times from both 30°C cultures and cultures shifted to 35°C. Labeled mycelia were then treated as described previously (7).

Electrophoresis. Protein samples were analyzed by electrophoresis on one-dimensional sodium dodecyl sulfate-polyacrylamide slab gels or two-dimensional gels (19) as described previously (7). Samples compared contained equal amounts of acid-precipitable radioactivity in protein.

RNA extraction and cell-free translation. RNA was extracted by the method of Lovett and Leaver (15), and polyadenylated [poly(A)] RNA was isolated and translated in the wheat germ system as described

previously (7). RNA was also translated in the rabbit reticulocyte lysate system (Amersham; 0.4 μg of poly(A) RNA was added to 10 μl of lysate containing 16 μCi of [^{35}S]methionine (Amersham). These samples were incubated for 90 min at 30°C, and products were analyzed electrophoretically on one-dimensional gels (after the addition of an equal volume of twofold-concentrated sample buffer) or on two-dimensional gels (after lyophilization of the sample and addition of lysis buffer [19].

RESULTS

Protein synthesis after heat shock. When exponentially growing cultures of *A. ambisexualis* were shifted from 30 to 35°C, the patterns of pulse-labeled proteins were distinctly altered (at 40°C the labeling of all proteins is greatly reduced). Several bands resolved by electrophoresis in sodium dodecyl sulfate showed an increased degree of labeling during a 20-min pulse, whereas a few were less labeled (Fig. 1, slots A and B). Among those having increased labeling were polypeptides marked in Fig. 1 at 78,000 daltons (78k), 70k, 46k, and 44k which we consider more extensively below. The time course of changes in protein synthesis was analyzed by using 20-min (Fig. 2, slots A through D) or 5-min (Fig. 3, slots A through E) pulses of [^{35}S]methionine after temperature shift. Increased labeling of several bands, including the 78k and 70k bands, was detected by 5 to 10 min. (Fig. 3, slot C). On the other hand, the increased labeling of the 46k and 40k bands was only barely detectible by 40 to 60 min (Fig. 2, slot D), but was distinct by 80 min (Fig. 1, slot B). Greater resolution of newly synthesized proteins was achieved by two-dimensional electrophoresis (Fig. 4). By 80 min at elevated temperature there was an increased synthesis of several major polypeptides, most of which are at the acidic end of the isolectic gels. Particularly noticeable was the increased labeling of clusters of spots having molecular weights of 78,000, 70,000, 46,000, and 44,000. These probably correspond to the 78k, 70k, 46k, and 44k bands identified on one-dimensional gels. There was also increased labeling of several other polypeptides including groups having molecular weights of 34,000 and 36,000 and the spots marked a, b, and c. All of the polypeptides which were more intensively labeled after heat shock were also being synthesized at the normal temperature. A few spots were less intensely labeled at the elevated temperature, but no spots disappeared.

Recovery from heat shock. Cultures which had been maintained at 35°C for 1 h were returned to the normal growth temperature of 30°C and pulse-labelled at various intervals. The elevated labeling of all polypeptides induced by heat shock was reduced substantially after 1 h (Fig. 2, slots E through H). More detailed analysis of the first 60 min of recovery (by using 5-min pulses of [^{35}S]methionine) indicated a temporally distinct pattern of recovery: labeling of the 70k protein was substantially reduced after 20 min at the lower temperature, whereas the 78k protein was still labeled at an elevated rate. The labeling of other heat shock proteins was reduced at various intervals up to 1 h. On closer examination of the 70k and 78k proteins at 1 h after initiation of recovery it was clear that the degree of labeling had decreased relative to other proteins to a lower level than that observed before heat shock (Fig. 2, slot E). Another recovery-associated phenomenon was the increased labeling of a polypeptide of 85,000 daltons within 5 min of the return to recovery temperatures. Increased la-

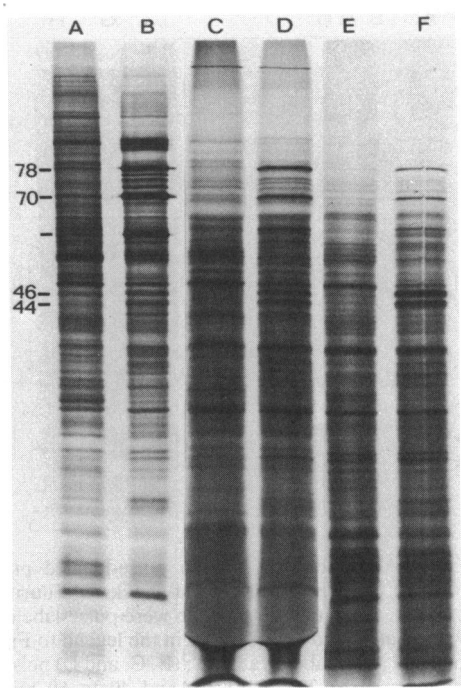

FIG. 1. Alterations of in vivo and cell-free translation products in *A. ambisexualis* mycelia before and after heat shock. An exponentially growing culture of *A. ambisexualis* was shifted from its normal growth temperature of 30°C to 35°C. Before the shift and at 1 h after incubation at 35°C, 1-ml samples were pulse-labeled for 20 min with [^{35}S]methionine for analysis of in vivo-labeled proteins (slots A and B). Poly(A) RNA was isolated and translated in rabbit reticulocyte lysate (slots C and D) and wheat germ (slots E and F) cell-free systems. Autoradiograms are shown of solubilized in vivo and cell-free translation products analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and taken from 30°C control cultures (slots A, C, and E) and cultures maintained at 35°C for 1 h (slots B, D, and F) (molecular weights indicated $\times 10^3$).

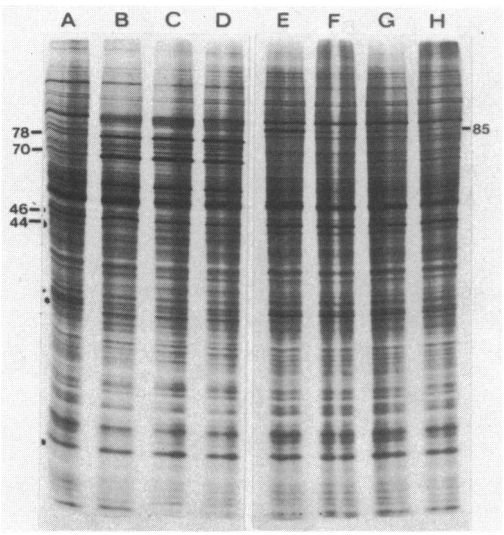

FIG. 2. Alterations of in vivo pulse-labeled proteins before heat shock, after heat shock, and during recovery. *A. ambisexualis* mycelia were pulse-labeled in vivo and analyzed as described in the legend to Fig. 1. Slots: (A) pulse labeled at 30°C; (B, C, and D) pulse-labeled from 0 to 20, 20 to 40, and 40 to 60 min, respectively, after shift to 35°C; (E, F, G, and H) pulse-labeled for 20 min at 1, 2, 3, and 4 h respectively, after return to 30°C (recovery) (molecular weights indicated $\times 10^3$).

beling of this polypeptide persisted up to 1 h (Fig. 3, slots F through I), but decreased to preshock levels by 2 h (Fig. 2, slots G through I).

Cell-free translation. To determine whether the changes in labeling of the heat shock-specific proteins were accompanied by similar changes in levels of translatable RNA, we isolated poly(A) RNA from control and heat-shocked mycelia, translated it in two cell-free systems, and then analyzed the products by electrophoresis on polyacrylamide gels. Many of the proteins, including the 78k, 70k, 46k and 44k species, which were more extensively labeled after 60 min at the elevated temperature, were coded for by mRNA's undergoing similar increases in translational activity as shown in both the rabbit reticulocyte lysate and wheat germ cell-free translation systems (Fig. 1). Analysis of these products by two-dimensional electrophoresis demonstrated that most of the spots which changed coincided with similarly positioned spots which changed during in vivo labeling (Fig. 5). As previously described (7), some polypeptides observed in vivo were not detectible in vitro and vice versa. This is probably due to posttranslational modification of proteins occuring in vivo, resulting in changes in position on two-dimensional gels. Such modifications may account for the more complex patterns of the clusters of 78k and 70k polypeptides observed in vivo. To characterize the populations of RNA available for translation at different times after heat shock and recovery, poly(A) RNA was isolated at various intervals after transfer to 35°C and also at various intervals after the return to 30°C (recovery) and was translated in a cell-free system. The pattern of labeling of the 70k and 78k bands on one-dimensional electrophoretic gels of cell-free translation products corresponded to that observed in vivo: increased levels were detectable at 10 min, and the increased level was maintained at 1 h (Fig. 6). The mRNA coding for the 46k and 44k bands increased mostly after 60 min as observed for protein synthesis in vivo.

The recovery pattern of the 78k and 70k bands of the cell-free translation products was also similar to that shown in vivo. After 20 min at 30°C labeling of the 70k band had decreased considerably, whereas labeling at the 78k band was still fairly high. After 60 min, labeling of the 78k band had decreased also. The 85,000-dalton recovery-associated protein seen in vivo was not detectable in the cell-free translation products (Fig. 6).

DISCUSSION

The elevation of the temperature of vegetatively growing cultures of *A. ambisexualis* from 30 to 35°C was rapidly followed by changes in protein synthesis. Several proteins were more

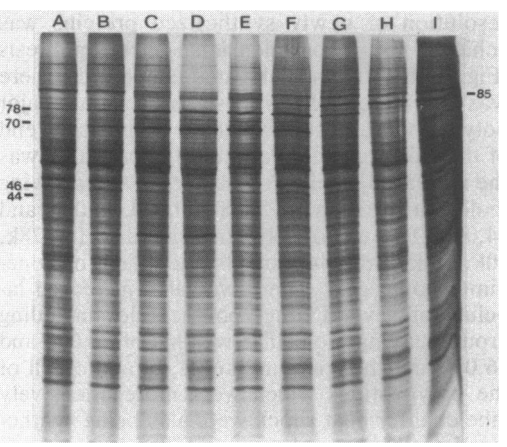

FIG. 3. Alterations of in vivo pulse-labeled proteins before heat shock, after heat shock, and during recovery (short pulse-labeling). *A. ambisexualis* mycelia were pulse-labeled for 5 min in vivo and analyzed as described in the legend to Fig. 1. Slots: A, pulse labeled at 30°C; (B, C, D, and E), pulse-labeled at 0, 5, 10, and 15 min, respectively, after shift to 35°C; (F, G, H, and I), pulse-labeled at 0, 20, 40, and 60 min, respectively, after return to 30°C (recovery) (molecular weights indicated $\times 10^3$).

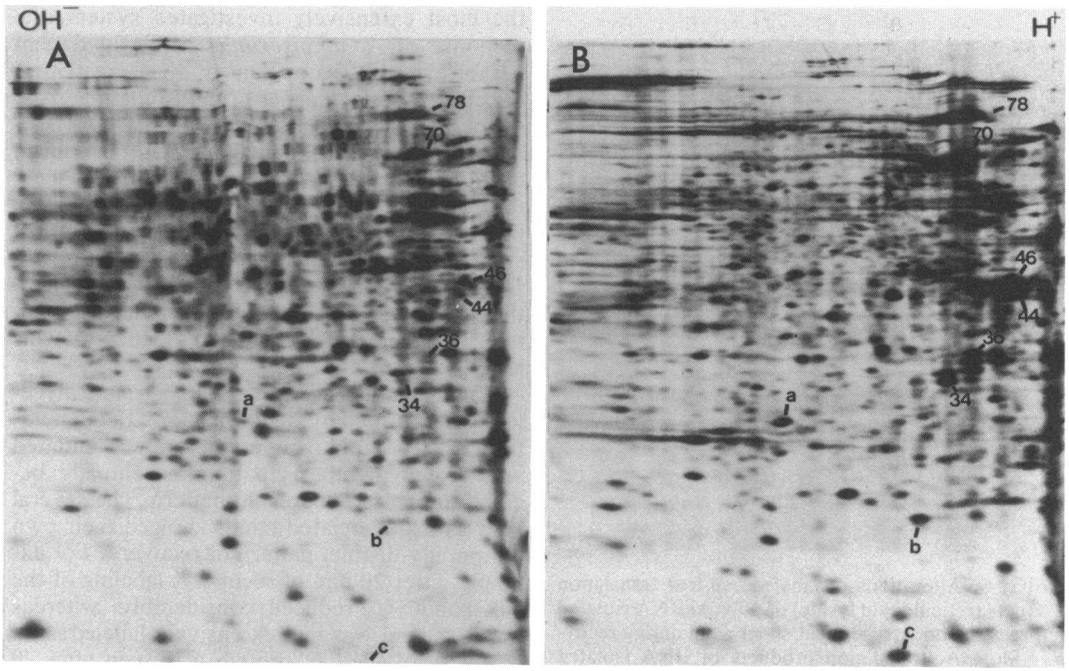

FIG. 4. Two-dimensional gel analysis of alterations of in vivo pulse-labeled proteins before and after heat shock. Gel A, Pulse labeled for 20 min at 30°C. Gel B, Pulse-labeled for 20 min after 1 h at 35°C (molecular weights indicated $\times 10^3$).

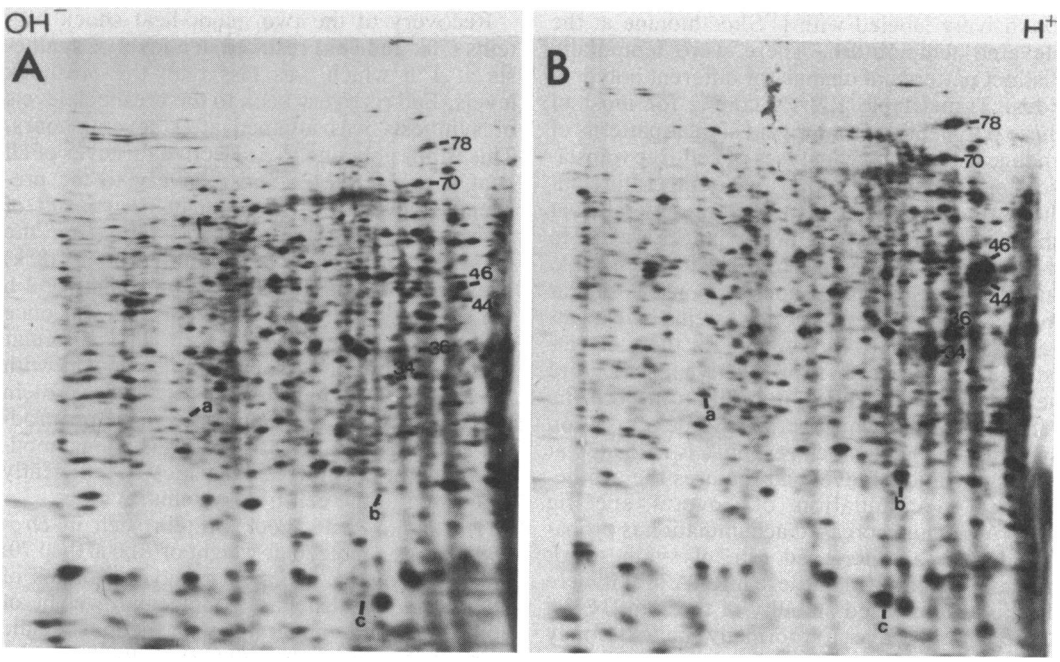

FIG. 5. Two-dimensional gel analysis of wheat germ cell-free translation products of poly(A) RNA isolated before and after heat shock. Gel A, Translation products of RNA isolated before heat shock. Gel B, Translation products of RNA isolated after 1 h at 35°C (molecular weights indicated $\times 10^3$).

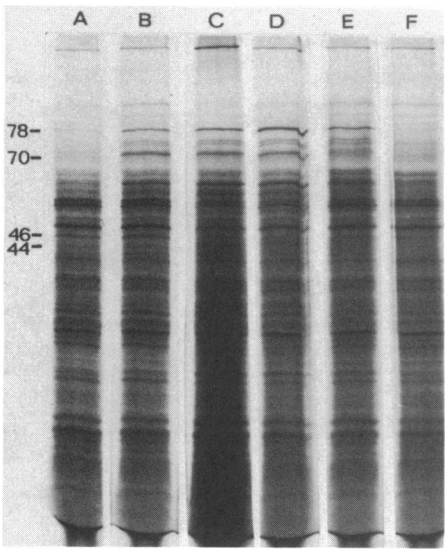

FIG. 6. Alterations of labeled cell-free translation products (reticulocyte lysate) of poly(A) RNA isolated before heat shock, after heat shock, and during recovery. Slots A, translation products of RNA isolated before heat shock; (B, C, and D), translation products of RNA isolated at 10, 40, and 60 min, respectively, after shift to 35°C; (E and F), translation products of RNA isolated at 20 and 60 min, respectively, after return to 30°C (recovery). (molecular weights indicated $\times 10^3$).

extensively labeled with [^{35}S]methionine at the elevated temperature. There were temporally distinct patterns of change for different polypeptides. Translatable RNA's coding for most of these polypeptides underwent similar patterns of change as shown by analyses of cell-free translation products. Thus, elevated temperature induces the increased synthesis of several heat shock polypeptides and these changes are due to the increased availability of translatable mRNA. In this respect the heat shock response is similar to that in all other eucaryotic systems investigated. Unless the mRNA's coding for these elevated proteins are stored in an inactive form (before heat shock), preventing translation in both the reticulocyte and wheat germ cell free translation systems, the heat shock response is mediated at a pretranslational level and requires the synthesis and accumulation of several specific mRNA's. This increased accumulation is probably due to an increased rate of synthesis or processing (or both) of these mRNA's. If it were due to an increased stability of these mRNA's, some would have to normally be extremely unstable to account for the very rapidly increased synthesis of some of the induced polypeptides.

Unlike the heat shock response of *Drosophila*, the most extensively investigated system, the response of *A. ambisexualis* to elevated temperature is not accompanied by a greatly reduced synthesis of most proteins characteristic of the normal temperature (18). In *A. ambisexualis* the synthesis of several proteins is reduced at the elevated temperature, and in this respect the response of *A. ambisexualis* resembles the heat shock response of other lower eucaryotes such as yeast or *Dictyostelium* (14, 16). In *Drosophila* the shutdown of normal protein synthesis during heat shock is translationally controlled (12, 23); we have no evidence for this kind of control in *A. ambisexualis*.

When after 1 h at 35°C *A. ambisexualis* mycelia were returned to the normal growth temperature, a fairly rapid recovery period was initiated and protein synthesis patterns eventually became similar to the preshift pattern. The several heat shock-associated bands showed their own temporally distinct pattern of recovery. For example, after 20 min of recovery, labeling of the 70k band was reduced considerably, whereas the 78k band was still extensively labeled. Full recovery of the 78k protein was seen after 40 min. A similar temporal recovery pattern for these two proteins was seen in the cell-free translation products, suggesting a temporally distinct decrease in abundance of translatable RNA for these two proteins. These fairly rapid changes may be due to selective turnover of mRNA's coding for these proteins.

Recovery of the two major heat shock proteins (70k and 78k) resulted in a level of synthesis at 1 h which was less than the preshock levels. Full recovery back to the preshock levels of synthesis was attained after several hours. This is possibly due to a selective turnover of all heat shock mRNA's and recovery to the preshock levels of synthesis might be a result of new transcription. For a short time after the initiation of recovery another protein (85k) showed an increased level of synthesis. By 2 h the pattern of synthesis of this protein was once again at the preshock levels. This particular protein is possibly associated in some way with the recovery mechanism. This particular protein was not seen in the cell-free translation products. It may be subject to posttranslational modification, or its mRNA may not be efficiently translated in the cell-free systems.

The major heat shock protein seen in *Drosophila* has a molecular weight of 70,000 (hsp 70) (18). One of the major heat shock proteins of *Dictyostelium* also has a molecular weight of 70,000 (14) and comigrates with the most acidic form of *Drosophila* hsp 70 (14). A 70,000-dalton protein is also observed as the major heat shock related product in sea urchin gastrulae (6). The most prominent heat shock protein in *A. ambi-*

sexualis also has a molecular weight of 70,000. Velazquez et al. (24) have reported that a major portion of the protein synthesized during heat shock in *Drosophila* is rapidly transported to the nucleus and becomes associated with the chromosomes. Since hsp 70 represents approximately 80% of total synthesis in *Drosophila*, some of this particular protein must migrate to the nucleus. Velazquez et al. (24) postulate that perhaps this protein is involved in stabilizing open chromatin during heat shock. This 70,000-dalton protein may have a common function in the heat shock response of many organisms, including *A. ambisexualis*.

Recent work has focused on investigation of the changes in gene expression in *A. ambisexualis* after different pathways of differentiation. Hormonally induced male sexual differentiation (the formation of antheridia) is accomplished with no detectable changes in RNA synthesis and minimal changes in protein synthesis (7, 20). The formation of asexual sporangia is accompanied by several changes in the pattern of protein synthesis which require transcription of new mRNA (Gwynne and Brandhorst, submitted for publication). The heat shock treatment described here induces considerably more prominent and rapid changes in gene expression in this oomycete than does induction of sexual or asexual differentiation. It is clear then that *A. ambisexualis* can undergo rapid and extensive changes in gene expression as part of a physiological response to stress. The fact that profound changes in RNA and protein synthesis do not accompany differentiation is thus not due to an inability of this primitive organism to alter its pattern of synthesis of RNA and protein.

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